ORIGINAL ARTICLE

Neural and Behavioral Responses to Tryptophan Depletion in Unmedicated Patients With Remitted Major Depressive Disorder and Controls

Alexander Neumeister, MD; Allison C. Nugent, PhD; Tracy Waldeck, PhD; Marilla Geraci, RN; Markus Schwarz, MD; Omer Bonne, MD; Earle E. Bain, MD; David A. Luckenbaugh, MA; Peter Herscovitch, MD; Dennis S. Charney, MD; Wayne C. Drevets, MD

Context: An instructive paradigm for investigating the relationship between brain serotonin function and major depressive disorder (MDD) is the response to tryptophan depletion (TD) induced by oral loading with all essential amino acids except the serotonin precursor tryptophan.

Objective: To determine whether serotonin dysfunction represents a trait abnormality in MDD in the context of specific neural circuitry abnormalities involved in the pathogenesis of MDD.

Design: Randomized double-blind crossover study.

Setting: Outpatient clinic.

Participants: Twenty-seven medication-free patients with remitted MDD (18 women and 9 men; mean±SD age, 39.8±12.7 years) and 19 controls (10 women and 9 men; mean±SD age, 34.4±11.5 years).

Interventions: We induced TD by administering capsules containing an amino acid mixture without tryptophan. Sham depletion used identical capsules containing hydrous lactose. Fluorodeoxyglucose F 18 positron emission tomography studies were performed 6 hours after TD. Magnetic resonance images were obtained for all participants.

Main Outcome Measures: Quantitative positron emission tomography of regional cerebral glucose utilization to study the neural effects of sham depletion and TD. Behavioral assessments used a modified (24-item) version of the Hamilton Depression Rating Scale.

Results: Tryptophan depletion induced a transient return of depressive symptoms in patients with remitted MDD but not in controls ($P<.001$). Compared with sham depletion, TD was associated with an increase in regional cerebral glucose utilization in the orbitofrontal cortex, medial thalamus, anterior and posterior cingulate cortices, and ventral striatum in patients with remitted MDD but not in controls.

Conclusion: The pattern of TD-induced regional cerebral glucose utilization changes in patients with remitted MDD suggests that TD unmasks a disease-specific, serotonin system–related trait dysfunction and identifies a circuit that probably plays a key role in the pathogenesis of MDD.

Arch Gen Psychiatry. 2004;61:765-773

Author affiliations are listed at the end of the article.

MAJOR DEPRESSIVE DISorder (MDD) has been associated with abnormally reduced function of central serotonergic systems by a variety of in vivo and postmortem findings. Relative to controls, patients with MDD have lower plasma tryptophan levels,[1,2] reduced cerebrospinal fluid 5-hydroxyindolacetic acid levels,[3,4] decreased platelet serotonin uptake,[5] and blunted neuroendocrine responses in challenge studies of different serotonin receptors.[6-12] Findings from brain imaging studies[13-16] and autoradiographic studies in tissue sections obtained post mortem[17,18] suggest widespread impairment of serotonergic function in depression with and without suicidality.

Tryptophan depletion (TD) has been used to test the hypothesis that decreased serotonin function is associated with MDD (for a review see Booij et al[19]). Administration of a tryptophan-free amino acid mixture of essential amino acids produces a rapid and substantial decrease in plasma tryptophan levels and a decrease in brain tryptophan, brain serotonin, and 5-hydroxyindolacetic acid levels in rats.[20,21] Studies in humans show profound decreases in plasma and cerebrospinal fluid tryptophan levels[22] and in cerebrospinal fluid levels of 5-hydroxyindolacetic acid[23,24] after oral administration of an amino acid mixture without tryptophan.

Table 1. Clinical and Demographic Characteristics of Unmedicated Patients With Remitted Major Depressive Disorder (rMDD) and Controls

Variable	rMDD Patients (n = 27)	Controls (n = 19)
Sex, F/M, No.	18/9	10/9
Age, mean ± SD, y	39.8 ± 12.7	34.4 ± 11.5
Age at onset, mean ± SD, y	23.8 ± 8.4	NA
Previous episodes, mean ± SD, No.	3.6 ± 2.6	0
Remission, mean ± SD, mo	40.4 ± 48.4	NA
Unmedicated time, mean ± SD, mo	39.1 ± 44.3	NA
Medication naïve, No.	4	19
Treatment history for last depressive episode, No.		
Selective serotonin reuptake inhibitors	11	NA
Tricyclic antidepressants	1	NA
Other (bupropion hydrochloride, venlafaxine hydrochloride, trazodone hydrochloride)	6	NA
Combination of the above	5	NA
Family history, No.		
Positive	23	0
Negative	4	19
Hamilton Depression Rating Scale score at baseline, mean ± SD	1.1 ± 1.2	0.5 ± 0.8

Abbreviation: NA, not applicable.

Findings that TD induces depressive symptoms in controls at high familial risk of depression[25-28] and in medication-free, remitted patients with a history of depression[29,30] but not in euthymic control subjects without familial risk of depression[24,25,29,31-36] suggest that either dysfunction within serotonergic systems or heightened sensitivity to reductions in serotonin transmission reflects a trait abnormality in depression.

The neural circuits associated with the core symptoms of depression and increased vulnerability for depression have not been precisely defined. Previous neuroimaging studies had design limitations, including small sample sizes, medication confounds, and illness heterogeneity. Such previous positron emission tomography (PET) studies[37] of TD-induced depressive symptoms in medicated, remitted individuals with depression showed that glucose metabolism decreased in the orbitofrontal cortex, ventrolateral prefrontal cortex (PFC), frontal polar cortex, pregenual cingulate cortex, and thalamus in patients who relapsed during TD but not in individuals who did not relapse. In addition, at baseline (before TD), patients who relapsed had increased metabolism in the amygdala relative to those who did not relapse. These data were largely consistent with subsequent studies[38,39] using PET with water labeled with oxygen 15 in patients with remitted MDD (rMDD) (most of whom were taking medication) that found that decreased tryptophan levels and transient return of depressive symptoms were associated with diminished cerebral blood flow in the ventral anterior cingulate cortex, orbitofrontal cortex, and caudate nucleus. These areas have also been implicated in depressive patients during an acute episode of depression[40] and thus may represent an important circuitry in MDD.

A major limitation of these investigations pertaining to identification of the primary serotonin-related neural circuits related to high risk of depression was that patients were studied while taking antidepressant medications. In this context, the purpose of the present study is to examine the behavioral and neural effects of TD in unmedicated patients with rMDD and controls. The present study extends previous research performed in MDD by revealing a trait abnormality of serotonin function in MDD in the context of specific neural circuitry abnormalities that may be involved in the pathogenesis of MDD.

METHODS

PARTICIPANTS

Participants were entered into the study after they received a full explanation of the purpose of the study and the study procedures and after they provided written consent as approved by the National Institute of Mental Health institutional review board. Twenty-seven remitted, unmedicated patients with a diagnosis of at least 2 past major depressive episodes on the basis of the *Structured Clinical Interview for* DSM-IV–*Nonpatient Version*[41] and 19 controls were included in the study (**Table 1**). Duration of depressive illness and number of episodes were estimated from the Past History of MDD addendum to the *Structured Clinical Interview for* DSM-IV–*Nonpatient Version*. Remission was defined as at least 3 months during which the patient did not take an antidepressant agent and had 24-item Hamilton Depression Rating Scale (HDRS) scores in the nondepressed range (<8).[42] Information about family history of mental illness (Axis I diagnoses) was obtained from the study participants for all first-degree relatives using the Family Interview for Genetic Studies.[43] Controls had no personal or family (first-degree relatives) history of psychiatric disorders. No use of psychotropic medications was allowed during the study. Participants were free of medical illness on the basis of history and results of physical examination, electrocardiography, and laboratory tests, including liver and kidney function tests, hematologic profile, thyroid function tests, urinanalysis, and toxicology. Pregnant and nursing women were excluded. Premenopausal women were studied during the follicular phase of the menstrual cycle. The menstrual phase was determined using plasma estradiol and progesterone concentrations, time since onset of last menses, and home urine ovulation kits to detect the mid-cycle luteinizing hormone surge (Clear Plan Easy; Whitehall Laboratories, Madison, NJ) to identify the time of ovulation within the index menstrual cycle.

TD PROCEDURE

Participants were enrolled into a double-blind, placebo-controlled crossover TD study and were randomly assigned to undergo either TD first and sham depletion (SD) second or SD first and TD second. To avoid carryover effects, depletion procedures were separated by at least 8 days. On the TD day, participants ingested 70 white capsules containing an amino acid mixture consisting of isoleucine (4.2 g), leucine (6.6 g), lysine (4.8 g), methionine (1.5 g), phenylalanine (6.6 g), threonine (3.0 g), and valine (4.8 g) at 7 AM. During SD, participants received 70 white capsules containing a total of 31.5 g of lactose at 7 AM. Patients were restricted from eating on day 1 of the study until completion of PET at about 4 PM, at which time they returned to unrestricted food intake. Study raters (M.G. and T.W.) were blind to whether the individual was a patient or a control. The effects of TD and SD were evaluated using mea-

sures of depression and measures of plasma total and free tryptophan concentrations.

Baseline clinical ratings were obtained at 7 AM and then 7 and 24 hours later using a modified (24-item) version of the HDRS. The items assessing insomnia, weight change, and diurnal variation were removed because they could not be meaningfully assessed on the days of the study.

ASSESSMENT OF PLASMA TOTAL AND FREE TRYPTOPHAN CONCENTRATIONS

Plasma total and free tryptophan levels were measured on each study day at baseline and then 5, 7, and 24 hours after intake of the capsules. Collected blood samples were immediately centrifuged for 15 minutes at 4°C and 3000 rpm. Plasma was frozen at –70°C until analyzed. Immediately after thawing, plasma proteins were precipitated by adding 20 µL of 70% perchloric acid to 400 µL of plasma, followed by centrifugation for 30 minutes at 20 000*g* at 4°C. For detection of total tryptophan, 100 µL of the supernatant was injected into the high-performance liquid chromatography system, leaving another 100 µL for a second injection. For the detection of free tryptophan, samples were filtered through a 10-kDa centrifugal filter device (Amicon Ultrafree-MC; Millipore Corp, Bedford, Mass) before injection. The system was calibrated using an external standard solution of tryptophan dissolved in a phosphate-buffered saline solution containing bovine serum albumin, 0.5 mg/mL. The standard solutions contained tryptophan concentrations ranging from 0.31 to 20.0 µg/mL for total tryptophan and 0.125 to 10.0 µg/mL for free tryptophan. (To convert tryptophan to micromoles per liter, multiply by 48.97.) For calibration, 10 µL of 70% perchloric acid was added to 200 µL of standard solution, thawed immediately before use, and handled in the same way as the plasma samples. The high-performance liquid chromatography system consisted of the Waters 2690 Separations Module (Waters Corp, Milford, Mass). The operational isocratic chromatographic conditions for this high-performance liquid chromatography system were set as follows: column temperature, 25.0°C; and flow rate, 1.0 mL/min. The mobile phase consisted of 2.5 g of sodium acetate, 100 mg of disodium EDTA, and 50 mg of sodium octyl sulfonate, which were dissolved in 2500 mL of deionized water and 150 mL of acetonitrile. A pH of 4.50 was reached by the addition of acetic acid to the buffer before acetonitrile was added. This solution was filtered through a 0.47-µm membrane filter and degassed before use. The analytical column was a 250 × 4–mm Supersphere 60 RP-select B, packed with C8 (LiChroCART 250-4; MERCK KGaA, Darmstadt, Germany).

Approximate run time after injection until detection of tryptophan was 10 minutes. A scanning fluorescence detector (Waters 474; Waters Corp) (λ excitation wavelength = 300 nm and λ emission wavelength = 350 nm) was used to detect tryptophan. The amount of a substance was obtained by the ratio of the peak height to the peak height of the calibration curve of the external standards. The tryptophan recovery evaluated by the amount of spiked vs nonspiked plasma after extraction was 90% to 100%. Intra-assay and interassay variations were less than 5%. The signal-to-noise ratio of the lowest standard (0.125 µg/mL) was greater than 30:1; the R^2 of the calibration curve was greater than 0.9992.

IMAGE ACQUISITION AND ANALYSIS

Magnetic resonance (MR) images were obtained for each participant using a 3.0-T scanner (Signa; GE Medical Systems, Waukesha, Wis) and a 3-dimensional magnetization-prepared rapid acquisition gradient-echo sequence (echo time, 2.982 milliseconds; repetition time, 7.5 milliseconds; inversion time, 725 milliseconds; voxel size, 0.9 × 0.9 × 1.2 mm) to provide an anatomic framework for analysis, partial volume correction of the PET images, and morphologic characterization so that individuals with anatomic abnormalities could be excluded.

Because the biochemical and behavioral effects of TD peak 5 to 7 hours after administration of the amino acid mixture, fluorodeoxyglucose F 18 (FDG) was infused approximately 6 hours after administration of the capsules containing either lactose or amino acids. Regional cerebral glucose utilization (rCMRGlu) was measured noninvasively by combining left ventricular chamber time-activity curve data with venous blood sample values to give the input function needed to calculate the metabolic rate. The left ventricular input function was obtained from dynamic PET imaging of the heart, with venous blood samples obtained concurrently with imaging after injection of 4.5 mCi (166.5 MBq) of FDG. Image data from the heart were acquired using a whole-body PET scanner (GE Advance; GE Medical Systems) in 2-dimensional mode for 35 minutes (ten 30-second frames and ten 3-minute frames). This was followed by a 10-minute emission and an 8-minute transmission brain scan 45 minutes after tracer injection. Cardiac slices were reconstructed, and 5 left ventricular slices were identified for region of interest (ROI) placement. The 0- to 5-minute frames were averaged to allow location of the left ventricular blood pool, whereas the 25- to 35-minute frames allowed identification of myocardial FDG uptake. Circular, 2-cm-diameter ROIs over the left ventricular chamber were positioned on each of the difference images (left ventricular image blood pool minus myocardial FDG uptake) such that spillover from the myocardium was minimized. An average left ventricular time-activity curve was obtained from the time-activity curves derived from the ROI in each of the 5 slices. The time-activity curve was extended in time to include the period of brain imaging by using venous blood sample values. The average values of the venous blood samples taken at approximately 25, 30, 35, and 50 minutes and the average left ventricular concentration during the 25- to 35-minute period were divided. This ratio was then used to scale the 50-minute venous sample concentration, which was then appended to the left ventricular curve, completing the input function used to generate parametric images of rCMRGlu.[44]

The effects of TD on rCMRGlu were assessed using whole-brain and MR image–based ROI analysis using image processing and analysis software (MEDx; Medical Numerics Inc, Sterling, Va). Whole-brain FDG uptake was measured using an MR image–based template. Primary ROIs were selected based on results of previous monoamine depletion studies[37,45] in medicated patients with rMDD, which revealed abnormalities in the orbitofrontal cortex, posterior cingulate cortex, medial thalamus, and dorsolateral PFC. Additional secondary ROIs were selected based on these regions showing alterations in functional imaging studies in untreated, symptomatic depressed patients[40] and consisted of the ventral striatum, pregenual cingulate cortex, subgenual cingulate cortex, ventrolateral PFC, and amygdala. All ROIs were defined a priori on an MR imaging template. These regions were placed on each patient's registered MR image. A binary mask of the gray matter was then used to ensure that only gray matter pixels were included in the analysis. Regions were then transferred to the coregistered PET images, and the mean metabolic activity was obtained for each ROI. The whole-brain measure was used to normalize the regional measures to factor out nonspecific global effects.

To explore TD-induced changes in regions outside the primary ROIs, the images were analyzed post hoc by voxel-by-voxel analysis using a statistical parametric mapping software package (SPM99; Wellcome Department of Imaging Neuroscience, London, England). The FDG images were coregistered to the MR images and spatially normalized to the standardized

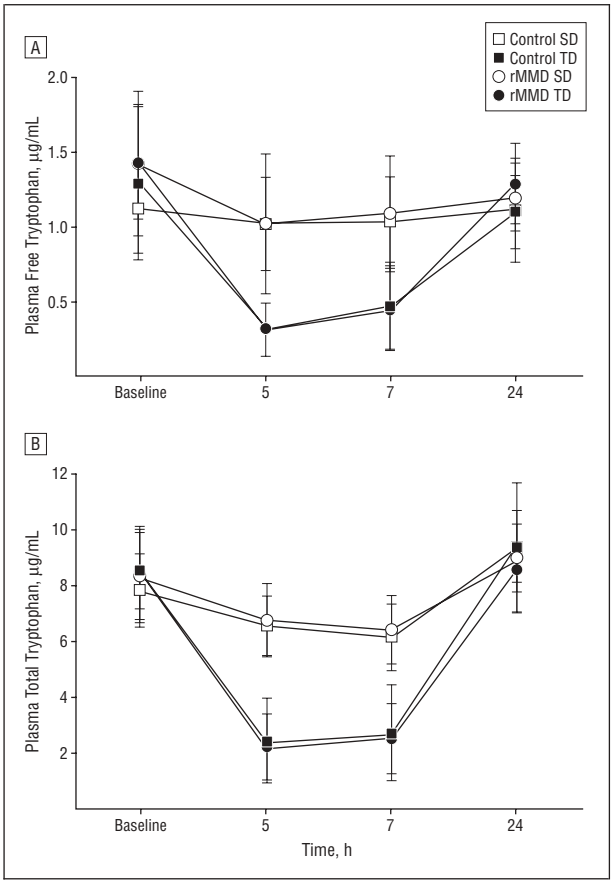

Figure 1. The effect of tryptophan depletion (TD) and sham depletion (SD) on mean plasma free (A) and total (B) tryptophan concentrations in patients with remitted major depressive disorder (rMDD) and controls. Tryptophan depletion, but not SD, reduced plasma levels of free tryptophan (treatment × time interaction: $F_{2.8,120.3}=74.35$; $P<.001$) and total tryptophan (treatment × time interaction: $F_{2.2,92.3}=163.32$; $P<.001$). Nadir values were found 5 to 7 hours after ingestion of the amino acid mixture, at the time the imaging studies were performed. To convert tryptophan to micromoles per liter, multiply by 48.97. Error bars represent SD.

space. Each image was then smoothed using a 12-mm gaussian kernel to compensate for errors in coregistration and normalization.

STATISTICAL ANALYSIS

Treatment (TD vs SD) × group (rMDD vs controls) repeated-measures analyses of variance were performed for each of the ROIs for whole-brain–normalized data. Greenhouse-Geisser *P* values were used to deal with concerns about the sphericity of the repeated-measures factors. Bonferroni-adjusted simple effects tests were used to evaluate the locations of differences. Four regions were considered a priori primary ROIs, and 5 regions were considered a priori secondary ROIs, so the α level was Bonferroni adjusted for the number of comparisons within each of these groups. All *P* values are 2-tailed and are reported before correction for multiple comparisons.

The same analysis of variance model and simple-effects tests were used, with the addition of a time factor for the total HDRS score and the HDRS sadness item score and for the plasma total and free tryptophan levels. The Fisher exact test was used to compare the relapse rates of patients and controls after TD. Return of symptoms was defined as a 10-point increase on the HDRS after baseline. Exploratory analysis of sex effects on rCMRGlu in the primary and secondary ROIs included adding sex as a factor in the analysis of variance model where treatment and group were factors. Follow-up tests were conducted in the same manner as those for the initial ROI analysis.

To study differences in rCMRGlu, the SPM analysis to visualize regional changes on a voxelwise basis between patients with rMDD and controls across conditions used a fixed-effects multigroup design incorporating HDRS scores as a covariate. The threshold for statistical significance was set at $P<.05$ corrected. Clusters with corrected values of $P<.05$ are reported. Voxels with uncorrected significance of $P<.001$ are reported if the voxels fell within the a priori, hypothesized primary and secondary ROIs.

An SPM analysis was also carried out to examine the relationship between mood state and rCMRGlu. Difference images obtained by subtracting normalized rCMRGlu images for the TD sessions from those for the SD sessions were compared, using the change in HDRS score as a regressor.

RESULTS

BIOCHEMICAL EFFECTS

Consistent with the literature, TD reduced plasma free and total tryptophan levels, whereas levels remained unaffected during SD (**Figure 1**). Plasma free tryptophan levels were reduced by 78% in patients with rMDD and by 76% in controls. Plasma total tryptophan levels were reduced by 74% in patients with rMDD and by 71% in controls. Order of test sessions, age, and sex did not affect the outcome.

BEHAVIORAL RESULTS

Tryptophan depletion, but not SD, was associated with a significantly greater increase in depressive symptoms, as reflected by an increase in HDRS total scores in patients with rMDD relative to controls (treatment × time × group interaction: $F_{1.2,51.0}=23.54$; $P<.001$) (**Figure 2**). Peak effects of TD on mood were found approximately 7 hours after administration of the amino acid mixture. The analysis for the sadness item (item 1) on the HDRS showed a robust increase during TD in patients with rMDD but not in controls, with no effects on mood in either group during SD (3-way interaction: $F_{1.2,53.5}=9.51$; $P=.002$). Sixteen (59%) of 27 patients with rMDD experienced a transient return of depressive symptoms during TD, whereas none met similar criteria during SD (Fisher exact test, $P<.001$). No control subject had depressive symptoms during TD or SD. Each patient with rMDD who experienced a transient return of depressive symptoms during TD reported feeling back to baseline on assessment at the follow-up (day 2) interview. Again, order of test sessions, age, and sex did not affect the outcome.

EFFECTS OF TD ON rCMRGlu

Primary ROI Analysis

No statistically significant between-group differences in whole-brain absolute rCMRGlu were found on the 2 test days. Thus, only normalized data are reported. Statistically significant between-group differences in response to

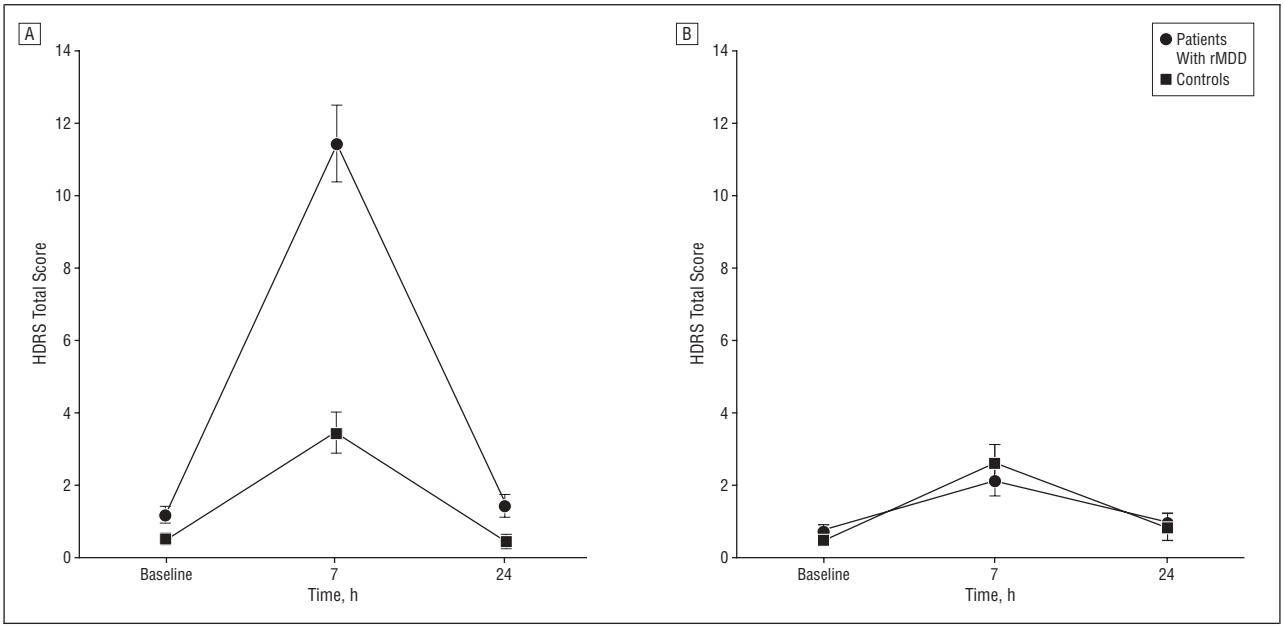

Figure 2. Mean (24-item) Hamilton Depression Rating Scale (HDRS) total scores show that tryptophan depletion (A), but not sham depletion (B), results in a transient return of depressive symptoms in patients with remitted major depressive disorder (rMDD). Peak behavioral effects were found approximately 7 hours after administration of the amino acid mixture. Error bars represent SD.

TD were found in the orbitofrontal cortex and the posterior cingulate cortex (**Figure 3**). We found a statistically significant group × treatment interaction for the lateral orbitofrontal cortex ($F_{1,40}$=7.36; P=.01), with an increase in rCMRGlu during TD relative to SD in patients with rMDD (P=.004) but not in controls (P=.32). A significant group × treatment interaction also was found in the posterior cingulate cortex ($F_{1,40}$=9.69; P=.003). Relative to SD, TD induced an increase in rCMRGlu in patients with rMDD (P=.03), whereas rCMRGlu decreased during TD relative to SD in controls (P=.04). In contrast, no significant group × treatment interactions were found in the medial thalamus and the dorsolateral PFC (**Table 2**). No statistically significant differences in rCMRGlu were found between patients with rMDD who had a return of depressive symptoms and those who did not experience a return of depressive symptoms during TD. Also, no differences in rCMRGlu were found at baseline (during SD) between patients with rMDD and controls.

Secondary ROI Analysis

Analyses of the secondary ROIs, implicated by functional imaging studies using PET in symptomatic depressed patients and postmortem studies, showed a significant group × treatment interaction in the ventral striatum ($F_{1,42}$=4.73; P=.04). After Bonferroni correction, this interaction was not significant (P=.20). Patients with rMDD had a statistically significant increase in rCMRGlu during TD relative to SD (P=.009), whereas rCMRGlu did not differ in controls between conditions. No significant group × treatment interactions were found for the pregenual cingulate cortex, subgenual cingulate cortex, ventrolateral PFC, or amygdala (Table 2).

Except for the posterior cingulate cortex, we did not see an effect of age or sex on rCMRGlu in any of the primary or secondary ROIs. Men with rMDD largely accounted for differences in the posterior cingulate cortex.

SPM ANALYSIS

The results of the voxel-based analysis of rCMRGlu comparing patients with rMDD and controls across treatments (TD vs SD) extended findings form the ROI analysis and showed significant between-group differences for the medial thalamus (z=4.18), anterior cingulate cortex (Brodmann area 32; z=3.97), and right putamen (z=3.81; P<.001 for all). The analysis of rCMRGlu for regions that showed a significant group × treatment interaction comparing patients with rMDD who showed a return of depressive symptoms relative to controls revealed significant (cluster-level corrected) between-group differences in the posterior cingulate cortex (Brodmann area 31; z=2.80; P=.003), left superior temporal cortex (z=2.75; P=.003), anterior cingulate cortex (Brodmann area 32; z=3.03; P<.001), and anterolateral PFC (z=2.99; P=.003). The rCMRGlu of patients with rMDD who showed a return of depressive symptoms differed significantly from that of patients with rMDD without symptom recurrence in the posterior cingulate cortex (Brodmann area 31; z=2.60; P=.005). We used a regression analysis to assess the effect of mood change on the TD and SD days in the rMDD group. Increasing depression scores were significantly (cluster-level corrected) correlated with increased rCMRGlu in the right precuneus (z=3.10) and the right putamen (z=3.04; P<.001 for both).

COMMENT

Medication-free, euthymic patients with a history of MDD differ statistically significantly from controls in their re-

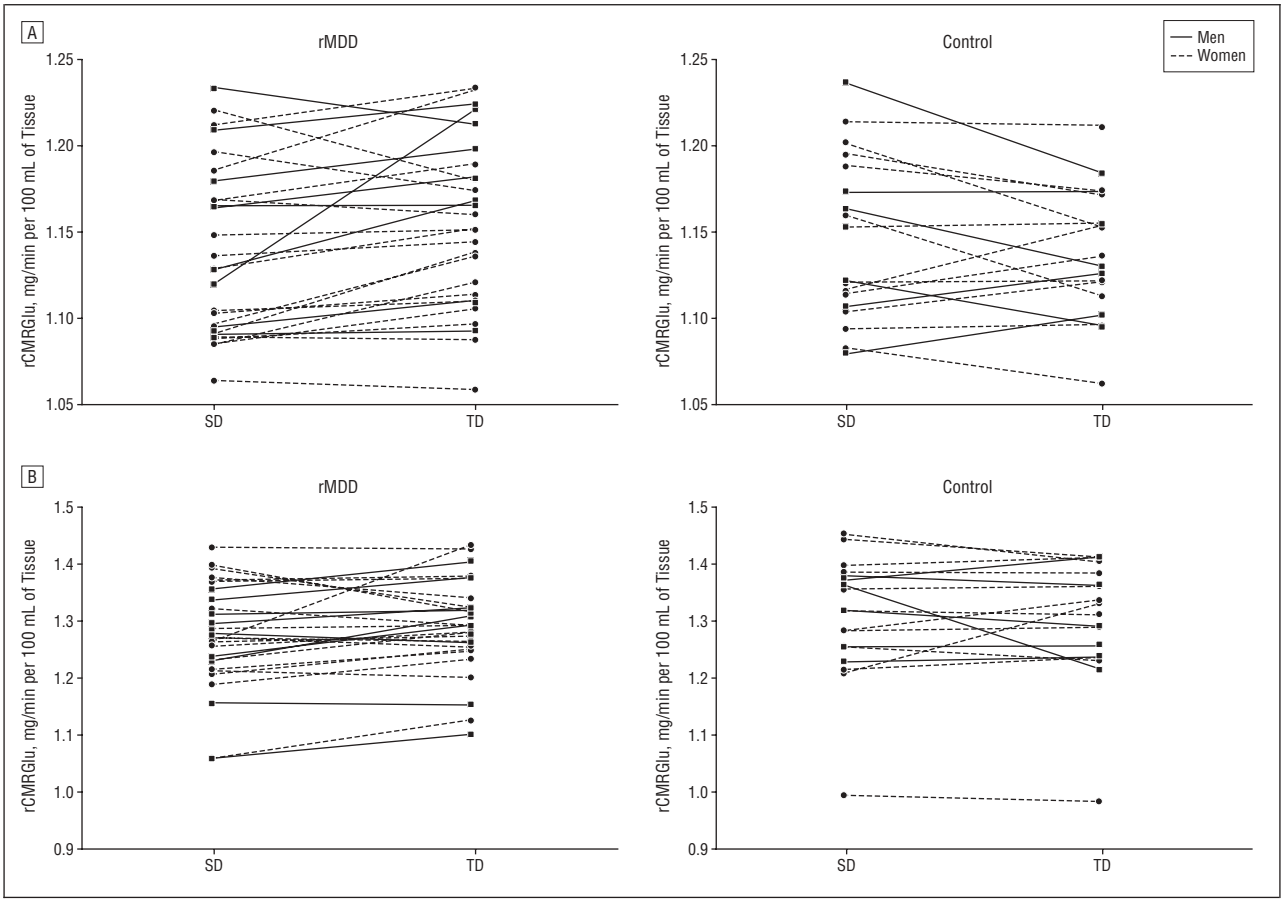

Figure 3. Regional cerebral glucose utilization (rCMRGlu) in unmedicated patients with remitted major depressive disorder (rMDD) (n=27) and controls (n=19) during tryptophan depletion (TD) and sham depletion (SD). A, In the orbitofrontal cortex, rCMRGlu increased during TD relative to SD in the rMDD group and was not significantly altered in the control group. B, In the posterior cingulate cortex, rCMRGlu increased during TD relative to SD in the rMDD group but decreased during TD relative to SD in the control group. No difference in rCMRGlu was found between patients with rMDD who showed a transient return of depressive symptoms during TD and those who were not affected by TD.

sponse to TD. Most individuals with a history of MDD experienced a transient return of depressive symptoms, whereas controls remained unaffected by TD. Neither group showed a depressive reaction during SD. These findings extend previous TD studies in patients with rMDD taking[37,46-49] and not taking[29,30] antidepressant medications by demonstrating the specificity of the depressiogenic effects of TD for patients with MDD and suggests that MDD is associated with a traitlike abnormality involving central serotonergic systems.

Our data further indicate that TD unmasks functional changes in the neural circuits implicated in the pathogenesis of MDD. In patients with rMDD relative to controls, TD was associated with increased rCMRGlu in the orbitofrontal cortex, anterior and posterior cingulate cortices, medial thalamus, and ventral striatum. Previous studies in unmedicated patients with MDD scanned during a spontaneous episode of MDD have consistently reported elevations of cerebral blood flow and glucose metabolism in patients with MDD relative to controls in these same regions (for a review see Drevets[40]). Previous imaging studies comparing patients with MDD during a spontaneous episode of MDD with controls were interpreted such that the elevated activity in these regions was mood dependent, partly because it was found also during experimentally induced sadness and anxiety in healthy individuals[50] and in symptomatic depressed patients in response to negative stimuli.[51] Antidepressant drug treatments result in a decrease in cerebral blood flow and metabolism toward normative levels in these regions.[51-54] The key finding that distinguishes the present study from previous similar work is that we did not find statistically significant differences in rCMRGlu between patients who had a return of depressive symptoms and those who did not during TD. This leads to the question of whether results from other functional neuroimaging studies really reflect current mood states or truly reflect an underlying "trait" abnormality in brain metabolism or blood flow associated with MDD. Our data suggest that TD unmasks a circuitry that truly represents a trait abnormality in MDD. Several researchers[55-57] have shown that the anterior and posterior cingulate cortices and the anteromedial PFC are involved in processing the affective salience of sensory stimuli and are involved in tasks that elicit emotional responses or require emotional evaluations. It can be hypothesized that these areas activate acutely during mood challenges, whereas dysfunctions in the limbic-cortical-striatal-pallidal-thalamic circuits represent a traitlike abnormality in MDD.

The magnitudes of the regional metabolic changes observed between the SD and TD conditions in the rMDD

Table 2. Change in Normalized rCMRGlu During Tryptophan Depletion (TD) and Sham Depletion (SD) in Patients With Remitted Major Depressive Disorder (rMDD) and Controls

	rCMRGlu Change, Mean ± SD, mg/min per 100 mL of Tissue				Statistical Analysis (ANOVA)*	
	rMDD		Controls			
Regions of Interest	TD	SD	TD	SD	F Value	*P* Value
Primary						
Lateral orbitofrontal cortex	1.16 ± 0.05	1.14 ± 0.05	1.15 ± 0.05	1.15 ± 0.06	7.36	.01†
Posterior cingulate cortex	1.29 ± 0.08	1.27 ± 0.09	1.28 ± 0.10	1.31 ± 0.11	9.69	.003†
Medial thalamus	1.37 ± 0.19	1.34 ± 0.19	1.29 ± 0.13	1.36 ± 0.16	5.10	.03
Dorsolateral prefrontal cortex	1.29 ± 0.06	1.29 ± 0.06	1.29 ± 0.06	1.29 ± 0.06	0.03	.88
Secondary						
Ventral striatum	1.35 ± 0.13	1.32 ± 0.12	1.31 ± 0.08	1.32 ± 0.08	4.73	.04
Pregenual cingulate cortex	1.15 ± 0.05	1.14 ± 0.06	1.14 ± 0.06	1.14 ± 0.06	0.01	.93
Subgenual cingulate cortex	1.12 ± 0.05	1.11 ± 0.06	1.11 ± 0.05	1.10 ± 0.06	0.06	.81
Ventrolateral prefrontal cortex	1.20 ± 0.06	1.19 ± 0.05	1.19 ± 0.04	1.18 ± 0.05	0.33	.57
Left amygdala	0.77 ± 0.11	0.78 ± 0.08	0.76 ± 0.10	0.75 ± 0.10	0.65	.42
Right amygdala	0.81 ± 0.10	0.82 ± 0.07	0.83 ± 0.08	0.82 ± 0.09	0.43	.52

Abbreviations: ANOVA, analysis of variance; rCMRGlu, regional cerebral glucose utilization.
*Results in the ANOVAs indicated group X treatment interactions.
†These results remain significant after Bonferroni correction for multiple comparisons.

sample ranged from approximately 2% to 3%. Similarly, the normalized regional metabolic values measured in patients with MDD during TD-induced depressive relapse were only 1% to 3% greater than the corresponding values in controls under either TD or SD conditions (Table 2). The magnitudes of these metabolic differences were thus modestly smaller than those of the metabolic differences found between currently depressed MDD cases and controls in the same regions, which have typically ranged from 3% to 6% when measured using ROIs placed using PET-MR image collocation.[54] The relatively smaller metabolic differences observed during TD-induced return of depressive symptoms in the present study may simply reflect the corresponding differences in depression severity between such studies. The severity of the depressive syndrome achieved during TD-induced return of depressive symptoms in the present study was mild, whereas previous studies comparing depressed MDD samples and controls imaged the depressed patients when they were in the moderately to severely depressed range. The relatively subtle changes in rCMRGlu we observed during TD-induced return of symptoms is nevertheless in the range expected for physiologic activation of tissue when taking into account the dilutional effects of our relatively large ROIs. During physiologic activation of cerebral tissue, the focal increases in metabolism are small (10%-40%) when measured directly over the area of maximal change.[58] In the present study, because we were unsure where the focal area of increase would occur within relatively broad areas of interest in individuals, metabolism was measured using low-resolution image analysis approaches to reduce type II error. In the MR image–based ROI analysis, the ROI size was substantially larger than the amount of cortex typically involved during physiologic activation, so the signal that we were trying to detect was substantially diluted. Similarly, in the SPM analysis, the images were blurred to a lower resolution to minimize the effects of misalignment errors that occur during the process of spatially normalizing the variable brain anatomy across individuals. As a result, the magnitudes of the metabolic changes detected by the SPM analysis were also diluted.

Tryptophan depletion did not induce significant metabolic differences between patients with rMDD and controls in the amygdala. Increased left amygdala metabolism has been reported during spontaneous episodes of MDD, and a positive correlation between increased metabolism and the severity of the depressive syndrome has been reported previously,[59] whereas lower right amygdala activity seems to predict a favorable treatment outcome.[52] Evidence suggests that normal serotonin function is important for proper amygdala function. Serotonin inhibits glutamate-evoked neuronal activity in the amygdala and modulates transmission of emotionally salient sensory information from the sensory cortices to the amygdala.[60] Reduced serotonin activity in MDD may disinhibit excitatory activity by reducing the stimulation of serotonin 1A receptors located on pyramidal cells, where they inhibit action potential formation, and of serotonin 3 receptors located on γ-aminobutyric acidergic interneurons, where they stimulate γ-aminobutyric acid release.[60-62] The failure of TD to induce significant changes in amygdala rCMRGlu may conceivably be related to the short-lived effects of TD, resulting in an only transient disruption of serotonin metabolism. Dysfunction in serotonin function sustained over a longer period and more pronounced depressive symptoms might be necessary to evoke changes in amygdala metabolism, as reported in the literature during spontaneous depressive episodes.

Our results differ somewhat from those of previous studies in medicated patients with rMDD who underwent mood challenges during scanning. Studies of cerebral blood flow[38] and rCMRGlu[37] during TD or during mood provocation with autobiographical memory scripts[63] reported diminished cerebral blood flow in the ventral anterior cingulate cortex, orbitofrontal cortex, and caudate nucleus[38,63] and decreased metabolism in the dorsolateral PFC, orbitofrontal cortex, and thalamus.[37] In the

latter study,[37] relapse-prone patients with rMDD had higher amygdala and PFC metabolism on the SD day than those who did not show depressive symptoms during TD. The present study in unmedicated patients with rMDD demonstrates, in contrast, areas with increased metabolism that agree with the literature in unmedicated symptomatic patients with MDD. These discrepancies in the direction of change and the circuitry involved may be explained by the interaction of the medications patients had been taking at the time of their studies with the TD and mood challenges vs the drug-free status in our patients. Another difference is the smaller dose of amino acids used in the present study to deplete tryptophan and the use of lactose during SD. We found reductions in plasma total and free tryptophan levels similar to the decrements reported by previous investigators.[22-27,29-39]

Our results add to functional imaging studies in untreated patients with MDD, lesion analyses, and postmortem studies that suggest that a well-characterized circuit seems to play a key role in the pathogenesis of MDD and probably represents a trait marker for MDD. Tryptophan depletion seems to be capable of unmasking this trait abnormality in MDD. As a potential phenotypic trait marker, TD seems to be a useful tool to study the genetic basis of MDD. An obvious next step in this type of investigation is to determine whether serotonin-related genes account for the robust differential neural and behavioral responses to TD in patients with rMDD and controls.

Submitted for publication January 6, 2004; final revision received February 24, 2004; accepted March 15, 2004.

From the Sections on Experimental Therapeutics and Pathophysiology (Drs Neumeister, Waldeck, Bonne, and Charney; Ms Geraci; and Mr Luckenbaugh) and Neuroimaging in Mood and Anxiety Disorders (Drs Nugent, Bain, and Drevets), Mood and Anxiety Disorders Program, National Institute of Mental Health, Bethesda, Md; the Department of Neurochemistry, University Hospital of Psychiatry, Munich, Germany (Dr Schwarz); and the PET Department, Clinical Center, National Institutes of Health, Bethesda (Dr Herscovitch). Dr Neumeister is now with the Department of Psychiatry, Yale University, New Haven, Conn.

This study was presented in part in abstract form at the 42nd Annual Meeting of the American College of Neuropsychopharmacology; December 9, 2003; San Juan, Puerto Rico.

Correspondence: Alexander Neumeister, MD, Section on Experimental Therapeutics and Pathophysiology, Mood and Anxiety Disorders Program, National Institute of Mental Health, North Drive, Bldg 15K, Room 200, Bethesda, MD 20892-2670 (neumeisa@intra.nimh.nih.gov).

REFERENCES

1. Cowen PJ, Parry-Billings M, Newsholme EA. Decreased plasma tryptophan levels in major depression. *J Affect Disord.* 1989;16:27-31.
2. Coppen A, Eccleston EG, Peet M. Total and free tryptophan concentration in the plasma of depressive patients. *Lancet.* 1973;2:60-63.
3. Koslow SH, Maas JW, Bowden CL, Davis JM, Hanin I, Javaid J. CSF and urinary biogenic amines and metabolites in depression and mania: a controlled, univariate analysis. *Arch Gen Psychiatry.* 1983;40:999-1010.
4. Asberg M, Thoren P, Traskman L, Bertilsson L, Ringberger V. "Serotonin depression": a biochemical subgroup within the affective disorders? *Science.* 1976; 191:478-480.
5. Healy D, Leonard BE. Monoamine transport in depression: kinetics and dynamics. *J Affect Disord.* 1987;12:91-103.
6. Mann JJ, McBride PA, Brown RP, Linnoila M, Leon AC, DeMeo M, Mieczkowski T, Myers JE, Stanley M. Relationship between central and peripheral serotonin indexes in depressed and suicidal psychiatric inpatients. *Arch Gen Psychiatry.* 1992;49:442-446.
7. O'Keane V, Dinan TG. Prolactin and cortisol responses to *d*-fenfluramine in major depression: evidence for diminished responsivity of central serotonergic function. *Am J Psychiatry.* 1991;148:1009-1015.
8. Cowen PJ, Charig EM. Neuroendocrine responses to intravenous tryptophan in major depression. *Arch Gen Psychiatry.* 1987;44:958-966.
9. Mann JJ, McBride PA, Malone KM, DeMeo M, Keilp J. Blunted serotonergic responsivity in depressed inpatients. *Neuropsychopharmacology.* 1995;13:53-64.
10. Meltzer HY, Maes M. Effects of ipsapirone on plasma cortisol and body temperature in major depression. *Biol Psychiatry.* 1995;38:450-457.
11. Heninger GR, Charney DS, Sternberg DE. Serotonergic function in depression: prolactin response to intravenous tryptophan in depressed patients and healthy subjects. *Arch Gen Psychiatry.* 1984;41:398-402.
12. Siever LJ, Murphy DL, Slater S, de la Vega E, Lipper S. Plasma prolactin changes following fenfluramine in depressed patients compared to controls: an evaluation of central serotonergic responsivity in depression. *Life Sci.* 1984;34:1029-1039.
13. Oquendo MA, Placidi GP, Malone KM, Campbell C, Keilp J, Brodsky B, Kegeles LS, Cooper TB, Parsey RV, van Heertum RL, Mann JJ. Positron emission tomography of regional brain metabolic responses to a serotonergic challenge and lethality of suicide attempts in major depression. *Arch Gen Psychiatry.* 2003;60: 14-22.
14. Mann JJ, Malone KM, Diehl DJ, Perel J, Cooper TB, Mintun MA. Demonstration in vivo of reduced serotonin responsivity in the brain of untreated depressed patients. *Am J Psychiatry.* 1996;153:174-182.
15. Malison RT, Price LH, Berman R, van Dyck CH, Pelton GH, Carpenter L, Sanacora G, Owens MJ, Nemeroff CB, Rajeevan N, Baldwin RM, Seibyl JP, Innis RB, Charney DS. Reduced brain serotonin transporter availability in major depression as measured by [^{123}I]-2β-carbomethoxy-3β-(4-iodophenyl)tropane and single photon emission computed tomography. *Biol Psychiatry.* 1998;44:1090-1098.
16. Drevets WC, Frank E, Price JC, Kupfer DJ, Holt D, Greer PJ, Huang Y, Gautier C, Mathis C. PET imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry.* 1999;46:1375-1387.
17. Lopez JF, Chalmers DT, Little KY, Watson SJ. Regulation of serotonin 1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus: implications for neurobiology of depression. *Biol Psychiatry.* 1998;43:547-573.
18. Mann JJ, Huang YY, Underwood MD, Kassir SA, Oppenheim S, Kelly TM, Dwork AJ, Arango V. A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. *Arch Gen Psychiatry.* 2000;57:729-738.
19. Booij L, Van der Does W, Benkelfat C, Bremner JD, Cowen PJ, Fava M, Gillin C, Leyton M, Moore P, Smith KA, Van der Kloot WA. Predictors of mood response to acute tryptophan depletion: a reanalysis. *Neuropsychopharmacology.* 2002; 27:852-861.
20. Curzon G. Relationships between plasma, CSF and brain tryptophan. *J Neural Transm Suppl.* 1979;15:81-92.
21. Gessa GL, Biggio G, Fadda F, Corsini GU, Tagliamonte A. Effect of the oral administration of tryptophan-free amino acid mixtures on serum tryptophan, brain tryptophan and serotonin metabolism. *J Neurochem.* 1974;22:869-870.
22. Williams WA, Shoaf SE, Hommer D, Rawlings R, Linnoila M. Effects of acute tryptophan depletion on plasma and cerebrospinal fluid tryptophan and 5-hydroxyindoleacetic acid in normal volunteers. *J Neurochem.* 1999;72:1641-1647.
23. Salomon RM, Kennedy JS, Johnson BW, Schmidt DE, Kwentus J, Gwirtsman HE, Ebert MH. Association of a critical CSF tryptophan threshold level with depressive relapse. *Neuropsychopharmacology.* 2003;28:956-960.
24. Carpenter LL, Anderson GM, Pelton GH, Gudin JA, Kirwin PD, Price LH, Heninger GR, McDougle CJ. Tryptophan depletion during continuous CSF sampling in healthy human subjects. *Neuropsychopharmacology.* 1998;19:26-35.
25. Benkelfat C, Ellenbogen MA, Dean P, Palmour RM, Young SN. Mood-lowering effect of tryptophan depletion: enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiatry.* 1994;51:687-697.
26. Klaassen T, Riedel WJ, van Someren A, Deutz NE, Honig A, van Praag HM. Mood effects of 24-hour tryptophan depletion in healthy first-degree relatives of patients with affective disorders. *Biol Psychiatry.* 1999;46:489-497.
27. Quintin P, Benkelfat C, Launay JM, Arnulf I, Pointereau-Bellenger A, Barbault S,

Alvarez JC, Varoquaux O, Perez-Diaz F, Jouvent R, Leboyer M. Clinical and neurochemical effect of acute tryptophan depletion in unaffected relatives of patients with bipolar affective disorder. *Biol Psychiatry.* 2001;50:184-190.
28. Neumeister A, Konstantinidis A, Stastny J, Schwarz MJ, Vitouch O, Willeit M, Praschak-Rieder N, Zach J, de Zwaan M, Bondy B, Ackenheil M, Kasper S. Association between serotonin transporter gene promoter polymorphism (5HTTLPR) and behavioral responses to tryptophan depletion in healthy women with and without family history of depression. *Arch Gen Psychiatry.* 2002;59:613-620.
29. Moreno FA, Gelenberg AJ, Heninger GR, RL, McKnight KM, Allen J, Phillips AP, Delgado PL. Tryptophan depletion and depressive vulnerability. *Biol Psychiatry.* 1999;46:498-505.
30. Smith KA, Fairburn CG, Cowen PJ. Relapse of depression after rapid depletion of tryptophan. *Lancet.* 1997;349:915-919.
31. Abbott FV, Etienne P, Franklin KB, Morgan MJ, Sewitch MJ, Young SN. Acute tryptophan depletion blocks morphine analgesia in the cold-pressor test in humans. *Psychopharmacology (Berl).* 1992;108:60-66.
32. Knott VJ, Howson AL, Perugini M, Ravindran AV, Young SN. The effect of acute tryptophan depletion and fenfluramine on quantitative EEG and mood in healthy male subjects. *Biol Psychiatry.* 1999;46:229-238.
33. Murphy FC, Smith KA, Cowen PJ, Robbins TW, Sahakian BJ. The effects of tryptophan depletion on cognitive and affective processing in healthy volunteers. *Psychopharmacology (Berl).* 2002;163:42-53.
34. Oldman A, Walsh A, Salkovskis P, Fairburn CG, Cowen PJ. Biochemical and behavioural effects of acute tryptophan depletion in abstinent bulimic subjects: a pilot study. *Psychol Med.* 1995;25:995-1001.
35. Voderholzer U, Hornyak M, Thiel B, Huwig-Poppe C, Kiemen A, Konig A, Backhaus J, Riemann D, Berger M, Hohagen F. Impact of experimentally induced serotonin deficiency by tryptophan depletion on sleep EEG in healthy subjects. *Neuropsychopharmacology.* 1998;18:112-124.
36. Yatham LN, Liddle PF, Shiah IS, Lam RW, Adam MJ, Zis AP, Ruth TJ. Effects of rapid tryptophan depletion on brain 5-HT(2) receptors: a PET study. *Br J Psychiatry.* 2001;178:448-453.
37. Bremner JD, Innis RB, Salomon RM, Staib LH, Ng CK, Miller HL, Bronen RA, Krystal JH, Duncan J, Rich D, Price LH, Malison R, Dey H, Soufer R, Charney DS. Positron emission tomography measurement of cerebral metabolic correlates of tryptophan depletion–induced depressive relapse. *Arch Gen Psychiatry.* 1997;54:364-374.
38. Smith KA, Morris JS, Friston KJ, Cowen PJ, Dolan RJ. Brain mechanisms associated with depressive relapse and associated cognitive impairment following acute tryptophan depletion. *Br J Psychiatry.* 1999;174:525-529.
39. Morris JS, Smith KA, Cowen PJ, Friston KJ, Dolan RJ. Covariation of activity in habenula and dorsal raphe nuclei following tryptophan depletion. *Neuroimage.* 1999;10:163-172.
40. Drevets WC. Neuroimaging studies of mood disorders. *Biol Psychiatry.* 2000; 48:813-829.
41. First MB, Gibbon M, Spitzer RL, Williams JBW. *Structured Clinical Interview for* DSM-IV–*Nonpatient Version.* New York, NY: Biometrics Department, New York State Psychiatric Institute; 1996.
42. Frank E, Prien RF, Jarrett RB, Keller MB, Kupfer DJ, Lavori PW, Rush AJ, Weissman MM. Conceptualization and rationale for consensus definitions of terms in major depressive disorder: remission, recovery, relapse, and recurrence. *Arch Gen Psychiatry.* 1991;48:851-855.
43. Maxwell ME. *Manual for the Family Interview for Genetic Studies (FIGS).* Bethesda, Md: Clinical Neurogenetics Branch, Intramural Research Program, National Insititute of Mental Health; 1992.
44. Moore DF, Altarescu G, Barker WC, Patronas N, Herscovitch P, Schiffmann R. White matter lesions in Fabry disease occur in "prior" selectively hypometabolic and hypoperfused brain regions. *Brain Res Bull.* 2003;62:231-240.
45. Bremner JD, Vythilingam M, Ng CK, Vermetten E, Nazeer A, Oren DA, Berman RM, Charney DS. Regional brain metabolic correlates of α-methylparatyrosine-induced depressive symptoms: implications for the neural circuitry of depression. *JAMA.* 2003;289:3125-3134.
46. Delgado PL, Charney DS, Price LH, Aghajanian GK, Landis H, Heninger GR. Serotonin function and the mechanism of antidepressant action: reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch Gen Psychiatry.* 1990;47:411-418.
47. Delgado PL, Miller HL, Salomon RM, Licinio J, Krystal JH, Moreno FA, Heninger GR, Charney DS. Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. *Biol Psychiatry.* 1999;46:212-220.
48. Aberg-Wistedt A, Hasselmark L, Stain-Malmgren R, Aperia B, Kjellman BF, Mathe AA. Serotonergic 'vulnerability' in affective disorder: a study of the tryptophan depletion test and relationships between peripheral and central serotonin indexes in citalopram-responders. *Acta Psychiatr Scand.* 1998;97:374-380.
49. Spillmann MK, Van der Does AJ, Rankin MA, Vuolo RD, Alpert JE, Nierenberg AA, Rosenbaum JF, Hayden D, Schoenfeld D, Fava M. Tryptophan depletion in SSRI-recovered depressed outpatients. *Psychopharmacology (Berl).* 2001;155: 123-127.
50. Schneider F, Gur RE, Mozley LH, Smith RJ, Mozley PD, Censits DM, Alavi A, Gur RC. Mood effects on limbic blood flow correlate with emotional self-rating: a PET study with oxygen-15 labeled water. *Psychiatry Res.* 1995;61:265-283.
51. Davidson RJ, Irwin W, Anderle MJ, Kalin NH. The neural substrates of affective processing in depressed patients treated with venlafaxine. *Am J Psychiatry.* 2003; 160:64-75.
52. Saxena S, Brody AL, Ho ML, Zohrabi N, Maidment KM, Baxter LR Jr. Differential brain metabolic predictors of response to paroxetine in obsessive-compulsive disorder versus major depression. *Am J Psychiatry.* 2003;160:522-532.
53. Drevets WC, Bogers W, Raichle ME. Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. *Eur Neuropsychopharmacol.* 2002;12:527-544.
54. Brody AL, Saxena S, Stoessel P, Gillies LA, Fairbanks LA, Alborzian S, Phelps ME, Huang SC, Wu HM, Ho ML, Ho MK, Au SC, Maidment K, Baxter LR Jr. Regional brain metabolic changes in patients with major depression treated with either paroxetine or interpersonal therapy: preliminary findings. *Arch Gen Psychiatry.* 2001;58:631-640.
55. Maddock RJ. The retrosplenial cortex and emotion: new insights from functional neuroimaging of the human brain. *Trends Neurosci.* 1999;22:310-316.
56. Dolan RJ, Fletcher P, Morris J, Kapur N, Deakin JF, Frith CD. Neural activation during covert processing of positive emotional facial expressions. *Neuroimage.* 1996;4(pt 1):194-200.
57. Reiman EM, Lane RD, Ahern GL, Schwartz GE, Davidson RJ, Friston KJ, Yun LS, Chen K. Neuroanatomical correlates of externally and internally generated human emotion. *Am J Psychiatry.* 1997;154:918-925.
58. Shulman RG, Rothman DL, Hyder F. Stimulated changes in localized cerebral energy consumption under anesthesia. *Proc Natl Acad Sci U S A.* 1999;96:3245-3250.
59. Drevets WC, Price JL, Bardgett ME, Reich T, Todd RD, Raichle ME. Glucose metabolism in the amygdala in depression: relationship to diagnostic subtype and plasma cortisol levels. *Pharmacol Biochem Behav.* 2002;71:431-447.
60. Stutzmann GE, McEwen BS, LeDoux JE. Serotonin modulation of sensory inputs to the lateral amygdala: dependency on corticosterone. *J Neurosci.* 1998; 18:9529-9538.
61. Mah EJ, Cunningham KA. Electrophysiological studies of serotonergic (5-HT) modulation of glutamate-evoked activity in amygdala neurons. *Abstr Soc Neurosci.* 1993;19:1554.
62. Morales M, Bloom FE. The 5-HT3 receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. *J Neurosci.* 1997;17:3157-3167.
63. Liotti M, Mayberg HS, McGinnis S, Brannan SL, Jerabek P. Unmasking disease-specific cerebral blood flow abnormalities: mood challenge in patients with remitted unipolar depression. *Am J Psychiatry.* 2002;159:1830-1840.